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## Genetic Characteristics and Relationship in Different Goat Populations of Indonesia Based on Cytochrome B Gene Sequences

<sup>1</sup>Suhendra Pakpahan, <sup>1</sup>Wayan Tunas Artama, <sup>1</sup>Rini Widayanti and <sup>2</sup>I. Gede Suparta

<sup>1</sup>Department of Biochemistry, Faculty of Veterinary Medicine, Gadjah Mada University, Yogyakarta, 55281, Indonesia

<sup>2</sup>Department of Animal Production, Faculty of Animal Science, Gadjah Mada University, Yogyakarta, 55281, Indonesia

*Corresponding Author: Wayan Tunas Artama, Department of Biochemistry, Faculty of Veterinary Medicine, Gadjah Mada University, Yogyakarta, 55281, Indonesia Tel: +62274-560865*

### ABSTRACT

Phylogenetic relationships and genetic variation among 9 Indonesian goat breeds were analyzed using cytochrome b gene sequences. The genomic DNA was isolated by gSYNC DNA Mini Kit (*Geneaid*) and amplified cytochrome b gene using PCR method with a pair of primer. Complete sequences of Cyt b genes of Indonesian goats is 1140 bp contained 19 variable sites and 8 haplotypes between Indonesian goats and *C. hircus* (D84201), when compared to *C. aegagrus* and *C. falconeri*, there are 59 variable sites. Phylogenetic analysis of haplotype in the combination with the goats from GenBank showed that Indonesian goats are grouped with *C. aegagrus*. Indonesian goat breeds have 4 amino acid units, which are different with *C. hircus*, at site 17, 191, 215 and 341th amino acids. The genetic distance score between Indonesian goat populations ranged from 0 to 0.007, while if compared to *C. aegagrus* ranged from 0.002 to 0.006 and to the *C. falconeri* ranged from 0.039 to 0.045. The relationship between subpopulation of Indonesian goats are very close. Indonesian goats have a closer genetic relationship with wild goat *C. aegagrus* than to *C. falconeri*.

**Key words:** Cytochrome b, goat, genetic distance, mitochondrial DNA, phylogeny relationship

### INTRODUCTION

Domestic goats (*Capra hircus*) have an economical, social and cultural values, which are very apparent to Indonesian farmers. In addition to producing meats, milk and skin, domestic goats are also used for religious rituals and traditional ceremonies (Mulyono and Sarwono, 2007; Chen *et al.*, 2005; Zhao *et al.*, 2011). Therefore, goats have a big potential to be developed in Indonesia. Goats are also livestock that are easy to breed and could adapt well to its environmental habitat (Budisatria *et al.*, 2008).

Livestock genetic resources are currently facing two challenges. On one side, the demand for livestock products are increasing in developing countries as estimated by Food Agriculture Organization (FAO), that the demand for milk and meat from livestock have increased twice than usual. On the other hand, livestock genetic resources are threatened because of the its aimless development (Ruane *et al.*, 2006).

Mitochondrial DNA (mtDNA) is a very important genetic marker and because of that it can only be passed down maternally without recombination and has a high mutation advancement (Muladno, 2006; Ratnayani *et al.*, 2007; MacHugh and Bradley, 2001). There were a lot of

studies based on mtDNA sequence analysis to study phylogenetic and evolutionary (Chen *et al.*, 2005; Joshi *et al.*, 2004; Bowling *et al.*, 2000; Zhao *et al.*, 2011). Bezoar (*C. aegagrus*) is the direct ancestor of domestic goats and Markhor (*C. falconeri*) is estimated to have given contribution to a number of Middle East goats descent (Mason, 1981). A recent study by Batubara (2011) about the genetic variation on six Indonesian goat breeds based on partial D-loop sequences obtained genetic variation of 0.014±0.002 and found 50 variable sites which consist of 19 haplotypes.

Takada *et al.* (1997) studied about the variation of cytochrome b mitochondria DNA (mtDNA) gene on Asian goats and concluded that the strongest candidate as the ancestor of domestic goats is the bezoar (*C. aegagrus*). Furthermore, phylogeographic mtDNA analysis reveals there are three different goat descendent lineage (estimated deviation >200.000 years ago) and suggested three different main origins of domestic goats (Luikart *et al.*, 2001). Sultana *et al.* (2003) had studied of the variation of Pakistani goats mtDNA and reported four different descendant lineage mtDNA called as A, B and C (previously reported) and a new lineage D. They concluded that there are at least four different wild *Capra* strain and may have been the source of domestic goats.

Indonesia has a high variety of natural resources. Natural resources are very important for the well-being of human lives so it needs to be conserved. Maintenance of genetic resources is crucial to conserve the genetic variation, so that the species can continue to survive. Therefore, characterization must be done to each existing organism. Goat's livestock resources in Indonesia currently consists of three groups, which are native livestock, import livestock and long adapted livestock (Batubara *et al.*, 2013). Indonesian goat livestock are divided into a number of subspecies that has different phenotype variety. This study is done to characterize Indonesian goat breeds based on cytochrome b gene sequences.

## MATERIALS AND METHODS

**Specimen collection:** This study used blood and hair follicle samples from individuals, which were divided into 9 subpopulation of Indonesian goat breeds (Table 1) namely Samosir goat (SAM), Muara goat (MUR), Gunung Sumatera goat (GS), Peranakan Etawah goat (PE), Jawarandu goat (JAW) and Kacang goat (KC), Lakor goat (LAK), Gembrong goat (GEM) and Marica goat (MAR). The goat samples were chosen using purposive sampling method, which first decided on the regency that are central production of native goats, then chose the sub-districts and villages. This study was conducted from September, 2014 to July, 2015.

**Mitochondrial DNA:** The blood and hair follicle samples were isolated using the gSYNC™ DNA Mini Kit (*Geneaid*). The primer was designed using Primer 3 online programs with the 4.0 version (<http://primer3.ut.ee>) based on data from a mitochondria genome sequence of *C. hircus* (KF952601). The goat mtDNA Cyt b primer sequences were as follows: SCF: 5'-GGAATCTAACCATGACCAAT-3'

Table 1: Sampling information for 9 populations of Indonesian goat breeds

Population	Province of origin	Island
Samosir goat	North Sumatera	Sumatera
Muara goat	North Sumatera	Sumatera
Gunung Sumatera goat	North Sumatera	Sumatera
Peranakan Etawah goat	D.I. Yogyakarta	Java
Jawarandu goat	D.I. Yogyakarta	Java
Kacang goat	East Java	Java
Lakor goat	Maluku	Maluku
Gembrong goat	Bali	Bali
Marica goat	South Sulawesi	Sulawesi

and SCR: 5'-GCTTCTTCCTTGAGTCTTAG-3'. The PCR conditions were: DNA template 3 µL, Kapa ready Mix 25 µL, each forward primer and reverse 1 µL and ddH<sub>2</sub>O in a total of 50 µL. The PCR amplification was conducted using a Infinigen Thermal Cycler according to program, initial denaturation at 94°C for 6 min, followed by 35 cycles, each consisting of 30 sec denaturation at 94°C, 45 sec primers annealing at 48°C, 90 sec elongation at 72°C, then a final for 5 min elongation at 72°C and storage at 4°C. The PCR product was visualized using 1% gel agarose (5 µL PCR products added with 2 µL loading dye). Electrophoresis was run on 100 V condition for 60 min and the amplification result could be seen on UV light. The purified PCR products were sequenced directly by 1st BASE Sequencing INT (Malaysia).

**Data analysis:** The length of the Cyt b gene sequences amplification product is 1140 bp for each individual and analyzed using MEGA 6.0 version software (Tamura *et al.*, 2013). The Cyt b gene sequences of Indonesian goats were aligned along with other *C. hircus* Cyt b sequences from GenBank using Clustal W in the MEGA program. Genetic variation analysis was seen based on the difference in cytochrome b gene sequences. Genetic distance was analyzed with the Kimura method using two parameters (Kumar *et al.*, 2001). Phylogenetic tree (Neighbor-joining tree) was made based on the Cyt b gene sequences with bootstrap testing 1000x repetition (Felsenstein, 1985) and Kimura 2-parameter method (Kimura, 1980).

Furthermore, to give a more complete information, several Cyt b gene sequences from domestic and wild goats were downloaded from GenBank and used as a comparator to Indonesian goats in order to see the genetic distance and estimates its origin. The access code is as follows: *Capra hircus* (D84201), *Capra aegagrus* (AB004069), *Capra falconeri* (AF034736) and some goat isolates from various countries namely Laos (AB044308), China (EU350133), Thailand (FJ556557), Korea (JX010746), India (DQ093614).

## RESULTS

**Polymorphism of cytochrome b gene complete sequences:** Cytochrome b gene amplification was done using a primer pair, namely SCF and SCR. That primer could amplify Cyt b gene completely that is 1140 bp. Nucleotide sequences were edited based on forward and reverse sequences and then compared with either *Capra hircus* (D84201), *Capra aegagrus* (AB004069) and *Capra falconeri* (AF034736). Analysis result showed there were 19 variable sites between Indonesian domestic goats refers to *C. hircus* and 59 variable sites refers to *C. aegagrus* and *C. falconeri*. There several unique sites that could be used as genetic identification on every Indonesian goat populations (Fig. 1). Nucleotide sequence, which is polymorphism is substituted (mutation). A number of sites could differentiate clearly between Indonesian goats with *C. hircus* (D84201) that is site 396, 397, 441, 447, 450, 572 and 721th. Those variable sites have similarities among of Indonesian goats, but clearly different with *C. hircus* (D84201).

Indonesia has a wild goat that live in the Sumatera's forests and mountains, namely Gunung Sumatera goat which existence is now threatened with extinction. DNA isolations are done by combining hair follicle. Based on the nucleotide sequence of Cyt b genes showed that there were several nucleotides differences with Indonesian domestic goats (site 144, 309, 594, 643, 852 and 1069th). If compared to *C. aegagrus*, Gunung Sumatera goat has a very similar sequence.

**Variety of amino acids based on Cyt b gene sequences:** The amino acid alignment result of translation from all Cyt b sequences with the comparator *Capra hircus* (D84201) obtained from

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Fig. 1: Analysis of genetic variations based on mtDNA Cyt b gene complete sequences of Indonesian goat populations refers to the *C. hircus*, *C. aegagrus* and *C. falconeri* from GenBank, SAM: Samosir goat, MUR: Muara goat, PE: Peranakan etawah goat, JAW: Jawarandu goat, KAC: Kacang goat, LAK: Lakor goat, GEM: Gembrong goat, MAR: Marica goat

380 amino acids. Indonesian domestic goats have 5 amino acids different to *C. hircus* namely amino acids 17, 191, 215, 342 and 357th (Fig. 2). Amino acid 191, 215 and 357th is a unique differentiator between Indonesian goats with *C. hircus* (D84201). Jawarandu goats have one amino acid different from several Indonesian goat breeds, which is the 17th amino acid where *C. hircus* (D84201) alanine (A) amino acid turns to phenylalanine (F). This amino acid can be used as Jawarandu goats' marker to differentiate it with other Indonesian goats.

According to Mason (1984) *C. aegagrus* or bezoar is a wild goat is that the direct ancestor of domestic goat and Markhor (*C. falconeri*) and has contributed to number of goat species in Asia. Based on complete Cyt b nucleotide sequences of Indonesian goats have similar genetics with *C. aegagrus* but many differences with *C. falconeri*. This proves that *C. aegagrus* (bezoar) is the ancestor of the native goats in Indonesia. Reviewed from the aspect of amino acid on Cyt b that Gunung Sumatera goat has similar amino acids with *C. aegagrus* that is it only has one different amino acid (the 17th amino acid) while compared with *C. falconeri* it has seven different amino acids.

The Genetic distance score between groups of Indonesian goats ranged from 0 to 0.007 (Table 2). The highest genetic distance is between Jawarandu goats with *C. falconeri* which is

Populasi	Site											
	1	1	2	3	3	3	3	3	3	3	3	3
	1	9	9	9	1	0	3	4	5	5	6	7
	7	8	0	1	5	4	6	2	7	9	9	7
<i>C. hircus</i>	A	I	T	G	T	V	H	A	Y	F	S	S
SAM 1	.	.	.	A	A	.	.	.	H	.	.	.
SAM 2	.	.	.	A	A	.	.	.	H	.	.	.
SAM 3	.	.	.	A	A	.	.	.	H	.	.	.
SAM 4	.	.	.	A	A	.	.	.	H	.	.	.
MUR 1	.	.	.	A	A	.	.	.	H	.	.	.
MUR 2	.	.	.	A	A	.	.	.	H	.	.	.
MUR 3	.	.	.	A	A	.	.	.	H	.	.	.
PE 1	.	.	.	A	A	.	.	.	H	.	.	.
PE 2	.	.	.	A	A	.	P	H	.	.	.	.
PE 3	.	.	.	A	A	.	.	.	H	.	.	.
JAW 1	P	.	.	A	A	.	.	.	H	.	.	.
JAW 2	P	.	.	A	A	.	.	.	H	.	.	.
JAW 3	P	.	.	A	A	.	.	.	H	.	.	.
KAC 1	.	.	.	A	A	.	.	.	H	.	.	.
KAC 2	.	.	.	A	A	.	.	.	H	.	.	.
KAC 3	.	.	.	A	A	.	.	.	H	.	.	.
KAC 4	.	.	.	A	A	.	.	.	H	.	.	.
LAK 1	.	.	.	A	A	.	.	.	H	.	.	.
LAK 2	.	.	.	A	A	.	.	.	H	.	.	.
GEM 1	.	.	.	A	A	.	.	.	H	.	.	.
GEM 2	.	.	.	A	A	.	.	.	H	.	.	.
GEM 3	.	.	.	A	A	.	.	.	H	.	.	.
MAR 1	.	.	.	A	A	.	.	.	H	.	.	.
MAR 2	.	.	.	A	A	.	.	.	H	.	.	.
MAR 3	.	.	.	A	A	.	.	.	H	.	.	.
GS 1	.	.	.	A	.	.	.	.	.	.	.	.
<i>C. aegagrus</i>	T	.	.	A	.	.	.	.	.	.	.	.
<i>C. falconeri</i>	.	V	A	A	.	M	Y	.	L	G	P	.

Fig. 2: Amino acid difference between the Indonesian goat populations (Compared to *C. hircus*, *C. aegagrus* and *C. falconeri* from GenBank), SAM: Samosir goat, MUR: Muara goat, PE: Peranakan etawah goat, JAW: Jawarandu goat, KAC: Kacang goat, LAK: Lakor goat, GEM: Gembrong goat, MAR: Marica goat

0.045. While, for some Indonesian goats breeds have a very close genetic distance with other breeds, but all Indonesian goat breeds have a quit distant genetic distance with *C. hircus* (D84201) this is caused by the adapted Indonesian goats with the environment for a long time. While, for the low genetic distance between Indonesian goat breeds, this is caused by the same ancestor of the Indonesian goats. The genetic distance between Indonesian goats with *C. aegagrus* is very close, ranged from 0.002 to 0.006 while with *C. falconeri* is quite far, around 0.039 to 0.044.

Domestic goats in Indonesia predicted that it originated from one species, that is kacang goat. This can be proved by the similarity in phenotype. All Indonesian goat breeds leads to Kacang goat phenotype. Domestic goats have adapted to their environment in where they live for a long period of time, so that will lead to change in the genetic materials. Based on Cyt b gene complete

Table 2: Estimates of evolutionary divergence over sequence pairs between groups

Correlation parameters	CH	SAM	MUR	PE	JAW	KAC	LAK	GEM	MAR	GS	CA	CF
CH		0.003	0.003	0.003	0.003	0.003	0.003	0.003	0.003	0.003	0.003	0.006
SAM	0.011		0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.002	0.002	0.006
MUR	0.011	0.002		0.001	0.001	0.001	0.001	0.001	0.001	0.002	0.002	0.006
PE	0.011	0.002	0.002		0.002	0.001	0.001	0.001	0.001	0.002	0.002	0.006
JAW	0.012	0.001	0.003	0.003		0.001	0.001	0.001	0.001	0.002	0.002	0.006
KAC	0.010	0.001	0.001	0.001	0.002		0.000	0.000	0.000	0.002	0.002	0.006
LAK	0.010	0.001	0.001	0.001	0.002	0.000		0.000	0.000	0.002	0.002	0.006
GEM	0.010	0.001	0.001	0.001	0.002	0.000	0.000		0.000	0.002	0.002	0.006
MAR	0.010	0.001	0.001	0.001	0.002	0.000	0.000	0.000		0.002	0.002	0.006
GS	0.008	0.006	0.006	0.006	0.007	0.005	0.005	0.006	0.005		0.001	0.006
CA	0.008	0.006	0.006	0.006	0.006	0.005	0.005	0.006	0.005	0.002		0.006
CF	0.042	0.044	0.044	0.044	0.045	0.043	0.043	0.043	0.043	0.039	0.039	

CH: *C. hircus*, CA: *C. aegagrus*, CF: *C. falconeri*, SAM: Samosir goat, MUR: Muara goat, PE: Peranakan etawah goat, JAW: Jawarandu goat, KAC: Kacang goat, AK: Gembrong goat, MAR: Marica goat, GS: Gunung sumatera goat, LAK: Lakor goat, GEM: Gembrong goat, number of base substitutions per site from averaging over all sequence pairs between groups are shown. Standard error estimate(s) are shown above the diagonal. Analyses were conducted using the Kimura 2-parameter model

sequences showed that genetic distance for each subpopulation of Indonesian goats are relatively close. Lakor goats have a very close genetic distance with Kacang goats while Indonesian domestic goats have a few far genetic distance with Gunung Sumatera goats. Indonesian domestic goats have a moderate genetic distance with goats from several Asian countries like; Laos, Korea, India and China (Fig. 3). This shows similarity in ancestry between domestic goats in the Asia Continent.

Phylogenetic tree was constructed using Neighbor Joining (NJ) method from Kimura 2-parameter module, where the total haplotype from all Indonesian goats and several goats obtained from GenBank could be separated roughly into two clusters (Fig. 3). All Indonesian goats forms one group with *C. aegagrus* and also with Chinese goat, Korean goat, Indian goat, Thai goat, Laos goat and *C. hircus* (D84201). This gives proven that Indonesian domestic goats come from the same ancestor with goats from other Asian countries.

## DISCUSSION

**Polymorphism of Cyt b gene sequences:** Mitochondria DNA Cyt b gene structure has been understood well, so this gene was widely used in phylogenetic study and this gene protein product's structure and function were well known (Giuffra *et al.*, 2000; Hiendleder *et al.*, 1998; Luikart *et al.*, 2001). Based on the different nucleotide sequence between subpopulation, so that specific genetic identification could be obtained to differentiate whether is each Indonesian goat or with *C. hircus* (D84201). Generally AT contents are always higher than GC contents in a Cyt b gene. This study is also consistent with that declaration, showing a proportion of 58:42 (Fig. 4), but slightly different from the study conducted by Chen *et al.* (2006), which examined 84 individuals Chinese goat breeds AT: GC proportion is 56:44.

The occurrence of mutation in Cyt b gene is medium compared to the D-loop region and other encoding genes. There were 19 variable sites found in a 1140 bp sequence of Cyt b gene. The genetic variations detected in this study were much fewer than those in mtDNA D-loop with 68 variable sites and 21 different haplotypes in a 1212 bp sequence (Pakpahan *et al.*, 2015). Batubara (2011) examined in 30 goats of six subpopulation partially sequenced the mtDNA D-loop found 50 variable sites in 879 bp amplification length. The mentioned variable area consist of 21 mutation insertion sites and 29 tranversions mutation sites. This proves that Cyt b gene is more conserved compared with D-loop non-coding region.



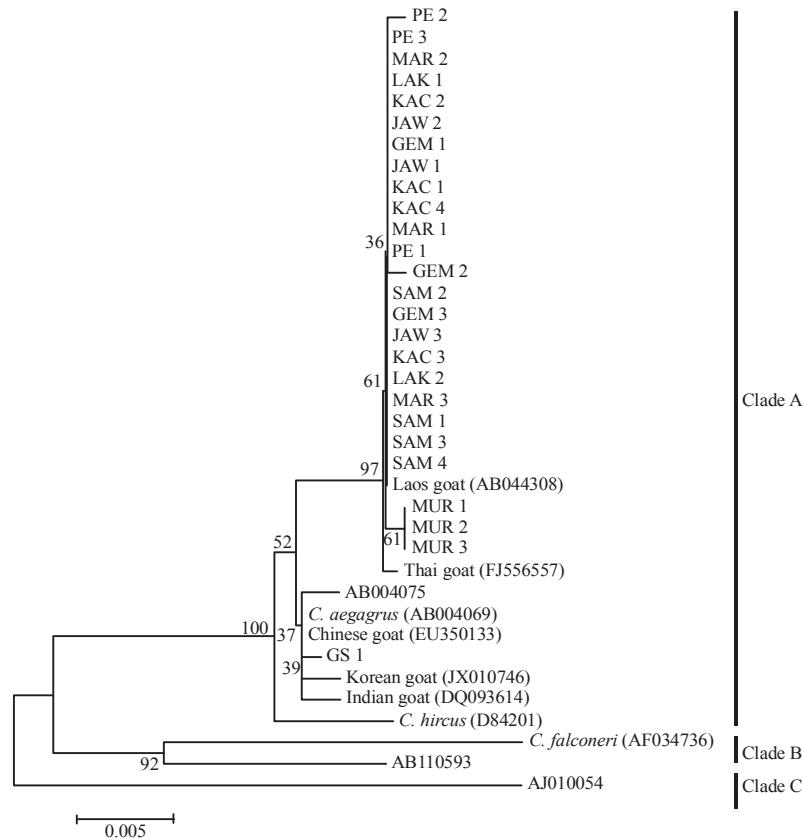


Fig. 3: Phylogenetic tree constructed from the neighbor-joining method using the Kimura 2-parameter model. The number at the branch node indicates the percentage occurrence in 1000 bootstrap replications. The bar scale indicates the genetic distance among Cyt b haplotypes

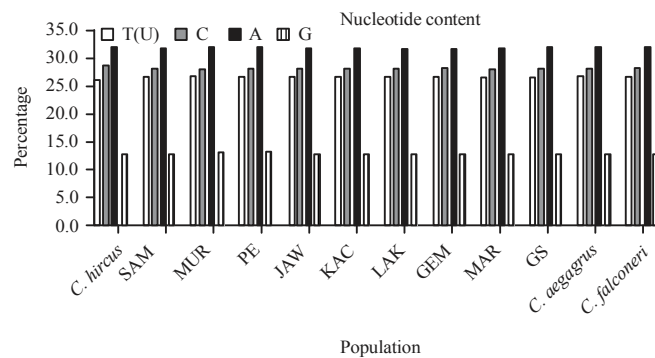


Fig. 4: Percentage of nucleotides T: Thymine, C: Cytosine, A: Adenine, G: Guanine in Indonesian goats based on Cyt b sequences compared to *C. hircus*, *C. aegagrus*, *C. falconeri*

**Relationship between population of Indonesian goat populations and several goats from GenBank:** Based on several phylogenetic analyses using previous mtDNA sequence, all Indonesian goats forms one clade with *C. aegagrus*, *C. hircus* and also with Chinese goat, Korean goat, Indian



goat, Thai goat, Laos goat. Takada *et al.* (1997) studied the variety of Asian goat genetics based on mtDNA Cyt b sequences and concluded that the strongest candidate as the domestic goats ancestor is *C. aegagrus*. Luikart *et al.* (2001) analyzed goat's mtDNA phylogeographic and stated that domestic goats all over the world originated from three different main "Ancestors". Sultana *et al.* (2003) mtDNA genetic variation of Pakistani goats and reported that Pakistani goats came from 4 different lineages that are called as A, B, C and D lineage. They concluded there are at least four strains from wild *Capra* that may be the source of modern domestic goats. Based on analysis of phylogenetic tree of haplotypes Cyt b sequences in this study, Indonesian goat populations are grouped with *C. aegagrus* and separated from *C. falconeri*. This proves that Indonesian goats have a relationship that is very close to *C. aegagrus*. Gunung Sumatera goat and several goats from other Asian countries formed one closer group. The genetic distance of Indonesian goat populations is closer to *C. aegagrus* than to *C. falconeri*.

Dissemination of goats in the Asian Continent is very wide, almost each country has goat livestock where that livestock has a little difference in phenotype because it has long adapted with its live environment. Indonesia goats form a group with Laos goat and Thai goat, it provides evidence that the Indonesian goats have a genetic distance, which is very close to the Laos goat and Thai goat. A number of authors state that Asian domestic goats have mostly originated from one ancestor which is *C. aegagrus* (Wang *et al.*, 2008; Liu *et al.*, 2006). Chen *et al.* (2005) stated that the Middle East has two types of wild goats (*C. aegagrus* and *C. ibex*) that possible had contributed to the origin and evolution of Asia's domestic goats, where the two goats could still be found to this day in Tibet and Inner Mongolia.

## **CONCLUSION**

Cytochrome b gene complete sequences could be used to see the genetic variation and relationship between Indonesian goats and also with goats from other countries (GenBank). Based on mtDNA Cyt b gene complete sequences, the genetic distance score between sub populations of Indonesian goats is around 0-0.007 and consist of 19 variable sites and also 8 haplotypes. This shows that every sup population of Indonesian goat breeds is very close. Indonesian goat breeds have a closer relationship to wild goat *C. aegagrus* than to *C. falconeri*. Indonesian goats have a close genetic distance with goats from several Asian countries (Laos, Korea, India and China).

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